

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 9/23/96	3. REPORT TYPE AND DATES COVERED Final		
4. TITLE AND SUBTITLE Ligands for stretch activated ion channels		5. FUNDING NUMBERS DAAL032-92-G-0014		
6. AUTHOR(S) Frederick Sachs				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) State University of New York at Buffalo Sponsored Programs Administration UB Commons, Suite 211 Amherst, NY 14228		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211		10. SPONSORING/MONITORING AGENCY REPORT NUMBER ARO 28775.7-L5		
11. SUPPLEMENTARY NOTES		19961021 159		
12a. DISTRIBUTION STATEMENT Approved for public release; distribution unlimited.				
13. ABSTRACT During tenure of the grant we have made tremendous strides in finding active ligands for the mechanosensitive channels. We developed a simple screen for the toxins based upon hypotonic swelling of GH3 clonal neurons which produced increases in internal Ca^{2+} levels that could be measured using Fura-2. Addition of active venoms would lead to a decrease in Ca^{2+} levels following swelling. Screening a variety of spider and scorpion venoms, we found that none of the scorpions tested (≈ 12) but one of the ≈ 8 spiders tested was able to block volume activated Ca^{2+} uptake. The raw venom also blocked stretch activated ion channels in <i>Xenopus</i> oocytes, chick heart cells and GH3 cells, and whole cell mechanical currents in chick heart cells.				
14. SUBJECT TERMS			15. NUMBER OF PAGES	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT UL	

Abstract:

During tenure of the grant we have made tremendous strides in finding active ligands for the mechanosensitive channels. We developed a simple screen for the toxins based upon hypotonic swelling of GH3 clonal neurons which produced increases in internal Ca^{2+} levels that could be measured using Fura-2. Addition of active venoms would lead to a decrease in Ca^{2+} levels following swelling. Screening a variety of spider and scorpion venoms, we found that none of the scorpions tested (≈ 12) but one of the ≈ 8 spiders tested was able to block volume activated Ca^{2+} uptake. The raw venom also blocked stretch activated ion channels in *Xenopus* oocytes, chick heart cells and GH3 cells, and whole cell mechanical currents in chick heart cells.

Publications arising from USARO support on this grant:

Chen, Y., S.M. Simasko, J. Niggel, W.J. Sigurdson, and F. Sachs (1996) Ca^{2+} uptake in GH3 cells during hypotonic swelling: the sensory role of stretch-activated ion channels. *Am.J.Physiol.* 270:C1790-C1798.

Diamond, S.L., F. Sachs, and W.J. Sigurdson (1994) The mechanically induced calcium mobilization in cultured endothelial cells is dependent on actin and phospholipase. *Arteriosclerosis and Thrombosis* 14:2000-2009.

Hu, H. and F. Sachs (1993) Effects of mechanical stimulation on embryonic chick heart cells. Upstate NY Cardiac Electrophysiology Society (Abstract)

Hu, H. and F. Sachs (1994a) Effects of mechanical stimulation on embryonic chick heart cells. *Biophys.J.* 66:A170(Abstract)

Hu, H. and F. Sachs (1994b) Characterizing whole-cell mechanosensitive currents in chick heart. *The Physiologist* 37:A9(Abstract)

Hu, H. and F. Sachs (1994c) Whole cell mechanosensitive current and its correlation with mechanosensitive channels in chick heart. Upstate NY Cardiac Electrophysiology Society (Abstract)

Hu, H. and F. Sachs (1995) Whole cell mechanosensitive currents in acutely isolated chick heart cells: correlation with mechanosensitive channels. *Biophys.J.* 68:A393(Abstract)

Hu, H. and F. Sachs (1996a) Mechanically activated currents in chick heart cells. *J.Membr.Biol.* 154: (in press)

Hu, H. and F. Sachs (1996b) Single-channel and whole-cell studies of mechanosensitive currents in chick heart. *Biophysical J.* 70:A347(Abstract)

Izu, Y.C., S. Simasko, and F. Sachs (1993) Calcium pathways involved in the hypotonicity-induced calcium increase in GH3 cells. *FASEB Journal* 7(4):A891(Abstract)

Izu, Y.C. and F. Sachs (1994) Hypotonic cell swelling induced intracellular calcium increase and whole cell currents in GH3 cells: a response to mechanical stimulus and a mechanism for volume regulation. Upstate NY Cardiac Electrophysiology Society (Abstract)

Nazir, S.A., D.J. Dick, F. Sachs, and M.J. Lab (1995) Effects of *G. spatulata* venom, a novel stretch-activated channel blocker, in a model of stretch-induced ventricular fibrillation in the isolated heart. Circ. 292:I-641, #3076(Abtract)

Niggel, J., H. Hu, W.J. Sigurdson, C. Bowman, and F. Sachs (1996) *Grammostola spatulata* venom blocks mechanical transduction in GH3 neurons, *Xenopus* oocytes and chick heart cells. Biophysical J. 70:A347(Abtract)

Ruknudin, A., F. Sachs, and J.O. Bustamante (1993) Stretch-activated ion channels in tissue-cultured chick heart. Am.J.Physiol. 264:H960-H972.

Ruknudin, A.M., H. Hu, and F. Sachs (1994) Extracellular ATP modulates stretch-activated channels in chick heart cells. The Physiologist 37:A6(Abtract)

Sachs, F. (1993a) Ligands for stretch activated ion channels. 6th Int.Symp.Nondestructive Eval.Mat. 124(Abtract)

Sachs, F. (1993b) Stretch activated whole cell currents - whole cell correlates. Trans.Bioelec.Repair Growth Soc.(Abtract)

Sachs, F. (1994) Modeling mechanical-electrical transduction in the heart. In V.C. Mow, F. Guliak, R. Tran-Son-Tray, and R.M. Hochmuth (eds): Cell Mechanics and Cellular Engineering. New York: Springer Verlag, pp. 308-328.

Sachs, F. (1995a) A low drift micropipette holder. Eur.J.Physiol. 429:434-435.(Abtract)

Sachs, F. (1995b) Mechanically sensitive ion channels: biological models for nanoscale stress sensors. *Nondestr.Charac.Mater.* 6 621-628.

Sachs, F., F. Qin, and P. Palade (1995) Models of Ca^{2+} release adaptation. *Science* 267:2010-2011.

Sachs, F. and H. Hu (1993) Stretch sensitive whole cell currents are alive and well. *Ann.Biomed.Eng.* 21:39(Abstract)

Sigurdson, W.J., F. Sachs, and S.L. Diamond (1993) Mechanical perturbation of cultured human endothelial cells causes rapid increases of intracellular calcium. *Am.J.Physiol.* 264:H1745-H1752.

Yang, X.C., and F. Sachs (1993) Mechanically sensitive, non-selective, cation channels. In D. Siemen and J. Hescheler (eds): *Non-selective ion channels*. Heidelberg: Springer-Verlag, pp. 79-92.

Zabel, M., B.S. Koller, F. Sachs, and M.R. Franz (1996) Stretch-induced changes in the isolated heart: importance of the timing of stretch and implications for stretch-activated ion channels. *Cardiovas.Res.* 32:120-130.

Izu, Y.C. (1994) A study of the sensory mechanism of cell volume regulation. PhD. Thesis, Biophysical Sciences, SUNY, Buffalo, NY.

Inventions:

Sachs, F., Bowman, C.B., Y. Chen-Izu. High Affinity Ligand for Mechanosensitive Ion Channels. SUNY disclosure submitted. Patent application submitted.

Degrees granted with grant support:

Y. Chen-Izu, 1994, SUNY Biophysics.

H. Hu, 1996, SUNY, Biophysics